ANALYSIS OF FORM REGULATION WITH THE AID OF ANÆSTHETICS.

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During the last year and a half the writer has carried on an extensive series of experiments on the influence of certain anæsthetics on the course and results of form regulation in *Planaria dorotocephala* Woodworth. Since the method has proved to be of considerable value and since it will be some time before the results of these and other experiments planned for the future can be presented in full, it seems desirable to present briefly and without extended comment the most important features of the work up to date as an illustration of the value of the method and the results to be attained by it.

In my experiments thus far I have used chiefly alcohol, ether and acetone-chloroform, known commercially as chloretone. Although alcohol is much less strongly anæsthetic than many other substances its action is in general similar in kind to that of the substances commonly known as anæsthetics. The results with the three substances mentioned are practically identical so far as the more general features are concerned, though various differences of detail appear, which need not be considered here. Incidentally, however, it may be noted that these differences in the effects of the different anæsthetics serve to some extent as a means of determining the specific differences in the effects of different anæsthetics on the metabolic processes: how far they will prove to be of value in this direction only the future can determine.

In my experiments various concentrations of the three substances have been employed, but the most characteristic results in general have been obtained with alcohol 1.5 per cent., ether 0.4–0.5 per cent., chloretone 0.025–0.0375 per cent. These concentrations cause a considerable deathrate, but the results in the pieces which do not die within the first week or ten days are very uniform, so far as pieces of the same size and from the same region of the body are concerned. Alcohol has been used to a

larger extent than either chloretone or ether in my experiments up to the present time because it has given characteristic results and because it has seemed desirable to obtain fairly complete data concerning the effect of a mild anæsthetic upon various regions of the body, on pieces of different size and age and in different physiological condition as a basis for comparative work with substances with stronger anæsthetic properties. It is my intention to extend the work to various other groups of substances which have a definite effect on metabolism. In most of the work of this kind the effect of only a short period of time in the given medium has been determined. It seems to me particularly desirable to determine more exactly the effects of continued existence in media of various constitution on development and regulation. By such means it should be possible to advance a step further in our analysis of the processes of differentiation, localization and growth.

I. Methods.

The method of use of the anæsthetics, all three of which are more or less volatile, is as follows: Not more than ten pieces of the planarian are placed in a Stender dish holding about 250 c.c. and with ground edge and cover with ground groove exactly fitting the edge. This dish is filled to three fourths of its depth with the mixture of the desired concentration, some of the fluid is poured over the under side of the cover so as to wet its surface and fill the groove, and the cover is placed in position at once. Several of these dishes are placed in larger glass jars with ground edges, a liter or more of the fluid is poured over them into the jar and it is at once sealed by means of a glass plate on which a ring of vaseline corresponding to the edge of the jar has been smeared. In this way decrease in concentration is avoided as far as possible. Furthermore the fluid is renewed at least every forty-eight hours and the mixture of the desired concentration is always made up immediately before using. It has been found desirable to keep the temperature as nearly as possible constant, except where its influence on the results was to be determined. In most of my experiments the temperature has ranged between 17° and 20° C. but rarely beyond these limits. Rise in temperature much beyond 20° C. increases the

death-rate and in temperatures below 15° C. the regulatory processes are considerably retarded and certain complicating features which result directly from low temperature appear. With fairly constant temperature and regular renewal of the fluid it has been possible to keep various individuals and pieces alive in 1.5 per cent. alcohol for several months, and death, when it occurred, was apparently the result of starvation. As will appear below, however, the length of life of the pieces and individuals varies greatly according to conditions.

II. Certain Features of the Organization of Planaria.

In order to make clear certain of the results presented below it is necessary to call attention to the fact that in *Planaria dorotocephala* (and in *P. maculata*) the posterior region of the body in all animals above a certain small size is specified to a greater or less extent as a second individual or zoöid, which, when it attains a certain degree of physiological independence, separates from the other part by an independent motor reaction and becomes a complete animal. As I pointed out some years ago,[1] the presence of this second zoöid is indicated by the regional differences in the regulatory power of short pieces taken in sequence. When we compare pieces not more than one tenth the length of the whole animal and as nearly as possible of equal length, we find that the ability to form a head decreases in each successive piece from the original head-region posteriorly, until at about the region of the old pharynx the power to form a head disappears entirely and the pieces remain headless. But at about the middle of the postpharyngeal region, *i. e.*, at the level where fission occurs, the power of head-formation reappears rather suddenly. In species like *Planaria simplicissima*, which do not undergo fission, there is no increase in the power of head-formation in the postpharyngeal region. Other regional differences which indicate the presence of a second zoöid in the posterior region of the body are described in the paper referred to above. As further evidence along the same line, I may add that I have been able to induce fission experimentally without feeding under conditions where it does

[1] Child, "The Relation between Regulation and Fission in *Planaria*," Biol. Bull., XI., 3, 1906.

not occur normally, *e. g.*, in animals reduced by starvation from a length of 15–18 mm. to a length of 6–7 mm., in young animals of 7–8 mm. in length, in the pieces resulting from the fission of large individuals, and again in the pieces resulting from the fission of pieces derived from fission.

It follows from these facts that the posterior end of the first zoöid lies somewhere near the middle of the postpharyngeal region in large individuals, and posterior to this level is a second young individual.

III. The General Effects of the Anæsthetics.

In the first place the regulatory processes are much retarded and the retarding effects are greater in small than in large pieces. The most favorable cases in the anæsthetics require at ordinary temperatures ten days or more to reach a stage which similar pieces in water attain in four or five days.

In a short paper[1] attention has already been called to the fact that the change in the proportions of the pieces is retarded to a much greater extent than the formation of new parts. As a matter of fact the increase in length and the decrease in width of the pieces, which is so characteristic a feature of the regulation in *Planaria* does not occur to any appreciable extent until the pieces have become sufficiently acclimated and have reached a sufficiently advanced stage of development to move about in a manner approaching the normal. In the paper above referred to I pointed out that this fact confirms the conclusions which I have already stated in previous papers, viz., that this change in proportion is largely the result of mechanical conditions connected with movement and locomotion.

In general the formation of the new tail is retarded to a much greater extent than that of the head. A small amount of new tissue forms on the posterior cut surface, but outgrowth to form the typical, tapering posterior end does not occur until the specimens begin to creep about. Apparently the movement and use of the posterior end is not only a factor in determining its shape, but also stimulates the growth of new tissue.

[1] Child, "The Regulatory Change of Shape in *Planaria dorotocephala*," Biol. Bull., XVI., 6, 1909.

The process of regulation ceases in the anæsthetic before the degree of development attained in water is reached. The head remains of smaller size in proportion to the size of the piece, the "auricles" on the sides of the head, which in this species are normally long and pointed, remain short and blunt, the new pharynx attains only a fraction of the size attained in water, and intestinal regulation, with the exception of the degeneration of the smaller intestinal branches in many cases, does not occur to any appreciable extent until the animals begin to move about actively.

These results are sufficient to show that the method affords a means of separating more or less completely in time certain regulatory processes which under normal conditions occur together, and thus of determining in some degree their relations to each other, and in the cases of change of shape and development of the tail their relation to movement and use of parts.

Another most interesting difference between the pieces in anæsthetics and those in water is the relation between regeneration in the stricter sense and redifferentiation. In certain pieces, which in water form the whole head back as far as the eyes by regeneration of new tissue, only the more anterior portions of the head are formed in this way in anæsthetics. In this respect alcohol and ether differ to some extent: in ether apparently less regeneration and more redifferentiation than in alcohol occurs, and in some cases in ether only the extreme tip of the head is regenerated, all other parts being the result of redifferentiation. A case of this kind is figured in my paper referred to above (9, Figs. 14–16). As regards this point the process of regulation resembles that occurring after long continued starvation, but the substitution of redifferentiation for regeneration is even more complete in the anæsthetics than after starvation.

IV. The Effect in Relation to the Size of the Piece.

In general a larger piece is necessary for the production of a whole, or anything approaching a whole, than in water. Within certain limits the death-rate increases as the size of the piece decreases. Regulation is retarded to a much greater extent in smaller than in larger pieces. In smaller pieces the formation

of a single median eye-spot instead of two, bilaterally symmetrical in position, is much more frequent than in larger pieces. With decrease in size all stages between the fully developed head — *i. e.*, as fully developed as it ever becomes in anæsthetic media — and headless pieces can be obtained.

All of these differences in regulation which are connected with differences in size can be observed in water, but in the anæsthetics they occur in much larger pieces than in water.

V. The Effect in Relation to Region of the Body.

The regional differences are similar in character to those observed in water but some of them are more marked. In the anæsthetics, for example, pieces from the middle region of the body, even those as long as one fourth of the whole length, almost always remain headless, while in water only very short pieces from the middle region fail to produce heads. Pieces one fourth the length of the body taken from the region immediately behind the old head almost always produce heads in the anæsthetic as well as in water. In short, it is almost impossible to inhibit head-formation in pieces from this region, though there is not the slightest difficulty in inhibiting it completely in pieces of the same size from the middle region. When we compare pieces of the same size taken in sequence from the anterior end posteriorly, we find that the ability to form a head disappears much more rapidly in anæsthetics than in water, but at the region of fission it appears again in both media and the pieces from the posterior region of the body, *i. e.*, the pieces of the second zoöid, so far as they survive — they are like pieces of younger animals in being more sensitive than pieces from other regions when first placed in the anæsthetic, and in becoming more rapidly and completely acclimated — produce a larger percentage of normal heads (that is, normal for the medium) than pieces from any other region.

Manifestly the comparison of pieces from different regions shows much more clearly than any series of experiments in water that the ability to form certain parts is present in very different degree in different regions of the body, and since the effect of the anæsthetics is to depress the metabolic processes or certain of them, the ability to form certain parts is apparently closely connected with the intensity of certain metabolic processes.

VI. The Effect on Localization and Number of Parts.

The localization of the pharynx in anæsthetics is different from its localization in water. In prepharyngeal pieces the pharynx appears nearer the posterior end and in postpharyngeal pieces, nearer the anterior end than in water. This difference in the localization of the pharynx means that the process of redifferentiation does not extend so far from the posterior end in prepharygeal pieces, or from the anterior end in postpharyngeal pieces as in water. Moreover, the shifting of the pharynx toward the middle, which occurs in each piece in water, is greatly retarded and in many cases never completed in the anæsthetics.

This change in the localization of a specific organ resulting from a change in the external medium is of considerable interest and has a bearing upon the problem of localization in general. Driesch has maintained that the localization of morphogenetic processes in "harmonious equipotential systems," of which he regards *Planaria* as an example, cannot be accounted for on a physico-chemical basis. In this case the facts certainly demonstrate that a change in the constitution of the medium, of such a character as to affect the metabolic processes, brings about a change in the localization of a specific organ, the pharynx.

Another case which involves not merely localization but number of parts as well, concerns the eye-spots. Normally two eye-spots, symmetrically placed, appear in *Planaria* in regulation as well as in ontogeny. As noted above, however, we find that short pieces in water, especially those from the middle region of the body, often give rise to only one median eye-spot. In fact the formation of a single eye-spot instead of two is the first indication of decreased or incomplete ability to form a head. If we compare pieces of different lengths with anterior ends at the same level of the body we find that with decreasing length of piece single eye-spots appear more frequently, until in pieces of a certain length perhaps only single eye-spots are formed. With still further decrease in the length of the pieces the heads with single eye-spots give way to a headless condition. It should be noted, however, that the relation between the length of the piece and the character of the result is by no means constant, but is dependent on the length of the animal, the physiological condition

and various other factors. In shorter individuals, for example, a relatively larger fraction, though an absolutely shorter piece, is necessary for a certain result similar to that produced by a relatively smaller fraction, though absolutely longer piece from a longer individual. Moreover, even in the same individual paired eyes appear in pieces of a certain length from the anterior region and the region of the second zoöid, while pieces of the same length from the middle region produce either single eyes or else remain headless. With continued starvation the length of a piece necessary to produce a given result as regards eyes increases.

With this brief statement of the results of experiments in water we may now turn to the consideration of the anæsthetics. In the anæsthetics single eyes appear in much longer pieces than in water. By preparing similar pieces of the proper length from a certain region of the body and placing part of them in alcohol, or ether, part in water, it is often possible to obtain 100 per cent. of single eyes in the anæsthetic medium and 100 per cent. of normal paired eyes in the water.

But in the anæsthetic a rather remarkable further change occurs very frequently, though I have never observed it with certainty in water. After some two weeks or more in the anæsthetic the single-eyed heads give rise to two additional eyes symmetrically placed in the normal position and slightly posterior to the single median eye already present. These three-eyed individuals are a characteristic feature of the experiments with anæsthetics. All the eye-spots persist as long as the animals live, whether they remain in the anæsthetic or are returned to water.

But still another feature must be noted. The longer pieces in the anæsthetic frequently produce two eyes in the normal position. In many cases such pieces give rise after two weeks or more to a second pair of eye-spots a short distance behind the first pair and, like them, symmetrically placed. Such individuals then possess four eye-spots, all of which persist during life.

Briefly then a very common effect of the anæsthetic is to increase the number of the eye-spots beyond the normal. It should perhaps be noted that these additional eye-spots are not mere fragments of pigment, but typical eyes, the pigment being

associated with the unpigmented area representing the sensory cells in each case. Frequently, however, in the four-eyed individuals the unpigmented areas as seen in dorsal view in the living animals are continuous between the anterior and posterior eye on each side. But the pigment spots are, so far as my observations go, always distinct and arise separately, not by division of a pigment mass already present. An attempt to consider the possible factors involved in these peculiar phenomena must be postponed until the data are presented in full. It may be said, however, that these cases of supplementary eye-formation suggest that the formation of a single eye or of a pair under normal conditions inhibits the formation of further eyes within a certain region of the head, while in anæsthetic media this correlative inhibiting effect is not sufficient to prevent the formation of new eyes as the animal becomes more and more fully acclimated and the head grows larger.

The relation between the formation of single median eyes or paired eyes and the length of the piece in anæsthetics and in water demonstrate very clearly that even the localization of such an organ as the eye-spot is dependent, not merely on the character of the tissues from which it is formed, but upon the organization of the whole piece of which it forms a part. Moreover, the fact that, other things being equal, a longer piece is necessary for the formation of paired eyes in the anæsthetic than in water indicates that the conditions or processes in other parts of the piece are less effective in the localization of the eyes in anæsthetic media than in water.

VII. The Effect on the Whole Animal.

When whole animals of large size (15–18 mm.) are placed in the anæsthetics the first effect besides the more or less complete cessation of locomotion is usually the loss of the pharynx, which is often extruded within forty-eight hours, though it may be retained and undergo degeneration in situ. Following its extrusion or degeneration a small new pharynx is usually slowly formed in the old pharyngeal pouch, but this never attains anything like the size of the original organ. Small young animals usually do not extrude the pharynx, and, so far as I have been able to determine, it does not degenerate when retained.

The heads of whole worms in anæsthetics gradually assume the shape characteristic of heads regenerated under the same conditions. The auricles decrease in length until scarcely visible, the head becomes smaller in proportion to the body and its outlines become more rounded.

In many cases, though by no means always, the large individuals begin after a few days in the anæsthetics to degenerate in the region representing the posterior end of the first zoöid, *i. e.*, the anterior half of the postpharyngeal region, and this degeneration results in the complete separation of a posterior portion corresponding to the second zoöid and an anterior portion consisting of the first zoöid minus more or less of its posterior end. In some cases the disintegration proceeds gradually in each piece after separation until the whole is disintegrated, but very commonly it ceases after the separation of the two parts, the surfaces heal and the posterior piece develops a new head as it would do after normal fission, while the anterior piece produces a small amount of new tissue at its posterior end. This process of disintegration is not due to an infection or to any other accidental condition in the medium, as is clearly shown by two facts: first, it increases in frequency with decreasing temperature. At low temperature practically every large individual separates into two parts while in room temperatures separation often occurs in less than half. And second, such disintegration and separation almost never occurs in individuals below a certain size, either in high or low temperatures. In short we find that the posterior region of the first zoöid in large individuals is subject to degeneration and disintegration in anæsthetics, and more frequently in low than in high temperatures, while in small individuals degeneration rarely or never occurs in this region.

I can account for these facts only as follows: In the large individual the first zoöid is approaching the limit of size and its posterior end being most distant from the centers of correlation, the cephalic ganglia, is approaching a condition of what we may call physiological isolation. When we place such individuals in the anæsthetics we decrease the effective distance of transmission of the nervous processes and the posterior region of the first zoöid becomes still further isolated. This region is incapable of form-

ing a new animal in anæsthetics, as we can readily show by isolating it physically, *i. e.*, by cutting it out and placing it in an anæsthetic medium, where it almost always degenerates. Consequently its condition while still attached to the other parts is comparable to its condition when physically isolated, *i. e.*, it is then physiologically isolated to such a degree that it behaves as if physically isolated and disintegrates. When we add the effect of low temperature to that of the anæsthetic, the physiological isolation is more certainly and more completely induced, consequently disintegration of this region is more frequent in low temperatures. This process of disintegration does not occur in small individuals because they are far below the limit of size and the posterior end of the first zoöid is not physiologically isolated in anæsthetics to any such degree as in the larger individuals.

VIII. Acclimatization.

If the individuals or pieces live for a week or more in the anæsthetic they became more or less acclimated and begin to move about and react in the usual manner, though very slowly and imperfectly. In many cases they continue to live for months, especially in alcohol. In ether and chloretone I have not as yet been able to keep them alive for so long a time in concentrations corresponding in their effect on regulation to those of alcohol employed for this purpose. By beginning with mixtures of low concentration and gradually increasing the concentration they can be acclimated to concentrations which otherwise kill them within a few hours. I have not thus far attempted to determine the limit of acclimatization, since my attention has been directed chiefly to other problems. It is of interest, however, to note that young animals become more readily and more completely acclimated to mixtures of given concentration than do older ones, although the younger individuals show a higher death-rate than the older when first placed in the anæsthetic. And finally individuals which have been reduced in size by starvation do not show the same power of acclimatization as young animals of the same size, but on the contrary, their ability to become acclimated decreases as starvation and the decrease in size continue. These facts bear upon various recent attempts at

interpretation of the reduction processes as reversals of development (Lillie, Schultz and others). It is evident from my experiments that the individuals reduced by starvation, although they may be simpler morphologically, than the full-grown individuals, are physiologically not younger but older than these.

IX. Length of Life.

In an extended series of experiments I have attempted to collect data concerning the length of life in anæsthetics of constant concentration, with the result that some conclusions of interest have been reached.

As noted above, young individuals of small size are more sensitive than older, larger, and die in greater numbers during the first two or three days, but if they survive this period they very commonly live longer than the larger older animals, since they become more completely acclimated. Pieces from the posterior region of the body of large animals and corresponding to the second zoöid behave in the anæsthetics like young animals.

Animals and pieces which have been well fed up to the beginning of the experiment live longer in anæsthetics than starving individuals and pieces. It is of interest to note that the further starvation advances, the earlier do the animals die in anæsthetics of given concentration.

Pieces from different regions of the body show very different degrees of resistance to the anæsthetics. Pieces from the anterior region without the head live much longer than pieces of the same length from the middle region. Short pieces including the old head die much earlier than longer pieces and their death is not due to reduction in consequence of starvation to the limit of existence, for they die long before such reduction occurs. In other series of experiments pieces have been allowed to begin the process of regulation in water and have been placed in the anæsthetic at various stages. If we compare long and short pieces with anterior ends at the same level of the body, we find in general that, as regulation proceeds, the short pieces, which have undergone a more extensive reorganization than the long pieces, behave more and more like young animals when placed in the anæsthetic, *i. e.*, they are more sensitive at first, but if they survive they become more

readily and more completely acclimated than the longer pieces and outlive them. In short the more extensive the regulatory reorganization of the piece the younger it is physiologically, and vice versa. These facts bear upon the general problems of age and "rejuvenation."

X. Conclusion.

The above brief statement of results includes only the more important features of the experiments, but is, I think, sufficient to demonstrate the value of the method and the possibility of its application to various problems.. I hope to extend the experiments with these and other substances and with other species, in order to obtain a broader basis for comparison. I scarcely need call attention to the possibilities of the method for the analysis of morphogenetic phenomena.

The results attained by anæsthetics can be approached more or less closely and in certain respects by decreasing the supply of oxygen, by low temperature and by starvation. In this respect my experiments are in accord with the hypothesis suggested by various authorities, viz., that anæsthetics inhibit to a greater or less extent the fundamental metabolic reactions or certain of them. We shall probably find, however, on further experiment that different anæsthetics act differently in certain respects as regards the morphogenic processes.

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